

MOLECULAR EPIGENETICS

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Cell culture-induced gradual and frequent epigenetic reprogramming of invertedly repeated tobacco transgene epialleles (Aleš Kovařík)

The ability of mature plant cells to regenerate a whole organism is probably the most remarkable growth attribute of plant cells that distinguishes them from mammalian cells. The basis of such a capacity in plants lies in the availability of undifferentiated cells that can subsequently differentiate into all the cell types present in a mature organism. The exact molecular processes involved in the maintenance and/or induction of cell undifferentiation in plants are still poorly understood. Here we tested the hypothesis that the dedifferentiation process induces epigenetic reprogramming of promoters influencing gene activity. Using a two component transgene system involving two epiallelic variants of the invertedly repeated silencing locus (1) we have studied stability of trans-silencing interactions in cell culture and regenerated plants (Fig. 1). In parental hybrids the posttranscriptionally but not transcriptionally silenced epiallele of locus 1 trans-silenced and trans-methylated target locus 2. Expression and methylation of both silenced (Lo1/Lo2) and non-silenced (Lo1E/Lo2) hybrids were stable over several generations in plants. However, in early Lo1E/Lo2 callus decreased expression of the nptII reporter gene was observed while the Lo1/Lo2 remained silenced. Analysis of small RNA species and coding region methylation suggested that the nptII genes were silenced by a PTGS mechanism in both cultures. Expression changes were correlated with changes in methylation status of the 35S promoter at the silencing locus 1: the PTGS variant in Lo1/Lo2 line acquired methylation while the TGS epiallele in Lo1E/Lo2 line showed reduced methylation compared to the parental plant. Bisulfite genomic sequencing of locus 1 revealed molecules with no, intermediate and high level of methylation (Table 1). These data indicated that a cell culture process brought two epialleles of the silencer locus to the same epigenetic ground characterized by high epiallelic diversity. In regenerated plants about 75% of Lo1E/Lo2 individuals returned to the original non-silenced phenotype while 25% of individuals were silenced. From Lo1/Lo2 callus, 25% of regenerated plants showed increased expression whereas 75% of individuals remained silenced. The results demonstrated sensitivity of transgenes containing inverted structures towards epigenetic changes imposed by cell culture. We propose that many examples of tissue culture-induced phenotypic variability might originate from epigenetic alterations at repeated loci influencing their transcription status.

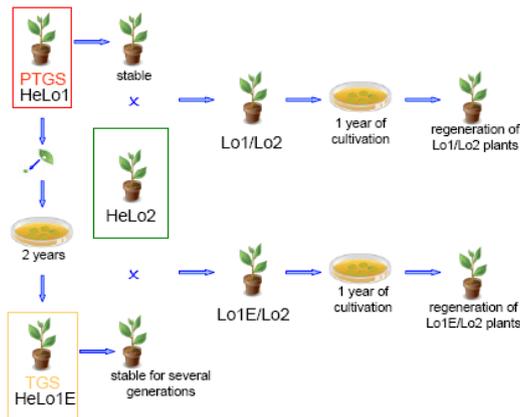


Figure 1: Scheme of the experimental strategy. HeLo1, plant line hemizygous for the transgenic locus 1, expression of the nptII reporter transgene silenced post-transcriptionally; HeLo1E, plant line hemizygous for the transgenic locus 1E, expression of the nptII reporter transgene silenced transcriptionally; HeLo2, plant line hemizygous for the transgenic locus 2, active expression of the nptII reporter transgene; Lo1/Lo2, posttranscriptionally silenced hybrid plant line combining locus 1 and locus 2; Lo1E/Lo2, hybrid plant line combining locus 1E and locus 2, active expression of the locus 2 nptII transgene.

Sample/methylation motif	mCG (%)		mCHG (%)		mCHH (%)	
	mean ^a	range ^b	mean	range	mean	range
Parental TGS Lo1/Lo2 plant	1	0-8	0	0-0	1	0-1
Disc of leaf (green tissue)	0	0-0	2	0-14	0	0-3
Early callus (4 weeks)	0	0-0	2	0-0	5	0-11
Advanced callus (12 months)	48	0-83	51	0-88	34	22-44
Non-silenced regenerant (#2)	90	83-100	75	57-100	34	22-44

Table 1: Summary of bisulfite sequencing analysis of the 35S promoter residing silencing locus (Lo1) in Lo1/Lo2 parental plant, derived calli and a regenerated plant.

^aPercent methylation at CG, CHG and CHH contexts was calculated from the clones (Fig. 5) and expressed as a mean.

^bMethylation homogeneity between the clones is indicated by a range.

Making a functional diploid: from polysomic to disomic inheritance (Roman Matyášek)

Polyploids may arise through chromosome duplication within a species (autopolyploidy) or in association with interspecific hybridisation (allopolyploidy). How do autopolyploid animal and plant species that have undergone one or more rounds of whole genome duplication become established in nature? An immediate difficulty concerns the transition from polysomic inheritance, where a chromosome can combine at meiosis with one of several partners (homologues), to disomic inheritance, where specific chromosome pairs form and segregate regularly. One little understood feature of polyploid speciation is the transition from polysomic to disomic inheritance, and much recent attention has focused on the role of pairing genes in this process. Using computer simulations we study the effects of mutations, chromosomal inversions, chiasma, neofunctionalisation, subfunctionalisation and selection on the evolution of disomic inheritance in a polyploid over 10,000 generations. We show that: (i) the evolution of pairing genes is not essential for the establishment of disomic inheritance, since genetic drift, coupled with a threshold for homologue pairing fidelity, is sufficient to explain the transition from polysomic to disomic inheritance; (ii) high rates of recombination increase the number of generations required for disomic inheritance to become established; (iii) both neofunctionalisation and subfunctionalisation speed up the transition to disomic inheritance. The data suggest that during polyploid species establishment, selection will favour reduced chiasma number and/or more focused distribution. The data also suggest a new role for subfunctionalisation in that it can drive disomic inheritance. The evolution of subfunctionalisation in genes across the genome will then act to maintain genes in syntenic blocks and may explain why such regions are so highly conserved.

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The histidine kinases CYTOKININ-INDEPENDENT1 and ARABI-DOPSIS HISTIDINE KINASE2 and 3 regulate vascular tissue development in Arabidopsis shoots

The development and activity of the procambium and cambium, which ensure vascular tissue formation, is critical for overall plant architecture and growth. However, little is known about the molecular factors affecting the activity of vascular meristems and vascular tissue formation. Here we show that the histidine kinase CKI1 and the cytokinin receptors AHK2 and AHK3 are important regulators of vascular tissue development in Arabidopsis shoots. Genetic modifications of CKI1 activity in Arabidopsis causes dysfunction of the two-component signaling pathway and defects in procambial cell maintenance. CKI1 overexpression in protoplasts leads to cytokinin-independent activation of the two-component phosphorelay, and intracellular domains are responsible for cytokinin-independent activity of CKI1. CKI1 expression is restricted to vascular tissues in inflorescence stems, and CKI1 forms homodimers both in vitro and in planta. Loss-of-function *ahk2* and *ahk3* mutants and plants with reduced levels of endogenous cytokinins show defects in procambium proliferation and an absence of secondary growth. CKI1 partially rescues *ahk2 ahk3* phenotypes in vascular tissue, while the negative mutation CKI1H405Q further accentuates mutant phenotypes. These results indicate that the cytokinin-independent activity of CKI1 and cytokinin-induced AHK2 and AHK3 are important for vascular bundle formation in Arabidopsis.

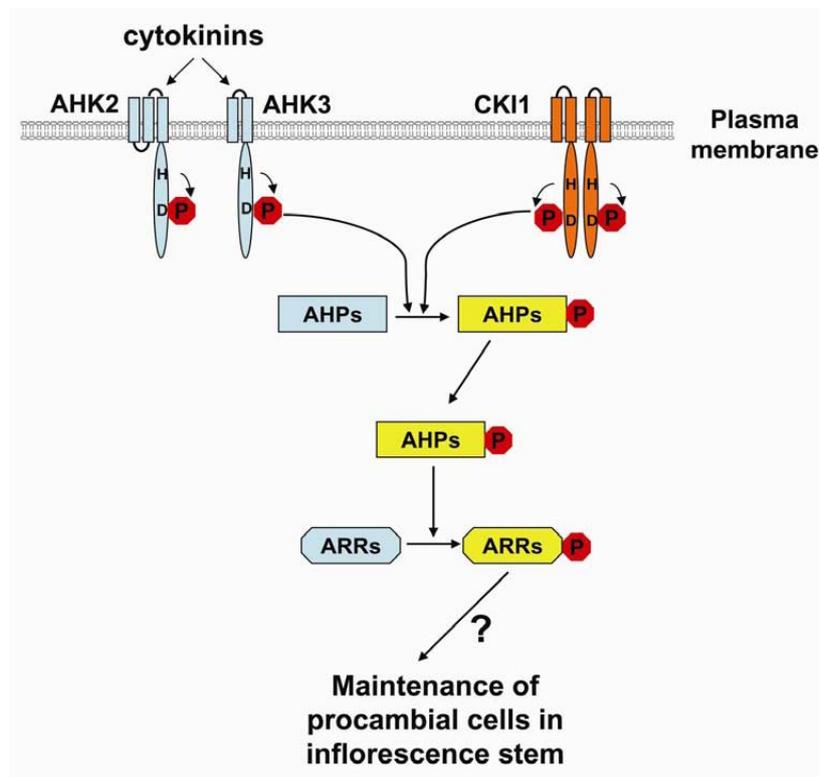


Figure 1: Interaction of cytokinin signaling pathway with histidine kinase CYTOKININ-INDEPENDENT1 (CKI1) in the vascular bundle development – a proposed model. We have shown that both the cytokinin-independent histidine kinase activity of CKI1 and cytokinin-activated histidine kinase activity of cytokinin receptors AHK2 and AHK3 are needed for regulation of procambium proliferation and/or the maintenance of its identity in Arabidopsis inflorescence stems.

Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux

Postembryonic de novo organogenesis represents an important competence evolved in plants that allows their physiological and developmental adaptation to changing environmental conditions. The phytohormones auxin and cytokinin (CK) are important regulators of the developmental fate of pluripotent plant cells. However, the molecular nature of their interaction(s) in control of plant organogenesis is largely unknown. Here, we show that CK modulates auxin-induced organogenesis (AIO) via regulation of the efflux dependent intercellular auxin distribution. We used the hypocotyl explants-based in vitro system to study the mechanism underlying de novo organogenesis. We show that auxin, but not CK, is capable of triggering organogenesis in hypocotyl explants. The AIO is accompanied by endogenous CK production and tissue-specific activation of CK signaling. CK affects differential auxin distribution, and the CK mediated modulation of organogenesis is simulated by inhibition of polar auxin transport. CK reduces auxin efflux from cultured tobacco cells and regulates expression of auxin efflux carriers from the PIN family in hypocotyl explants. Moreover, endogenous CK levels influence PIN transcription and are necessary to maintain intercellular auxin distribution in planta. Based on these findings, we propose a model in which auxin acts as a trigger of the organogenic processes, whose output is modulated by the endogenously produced CKs. We propose that an important mechanism of this CK action is its effect on auxin distribution via regulation of expression of auxin efflux carriers.